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ROLE OF IRRADIATION AND STATUS OF A-FETO PROTEIN, B_2-MICROGLOBULIN AND CARCINOEMBRYONIC ANTIGEN IN DIFFERENT STAGES OF ORAL CANCER PATIENTS

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ABSTRACT

Head and neck cancers are a major cancer burden in India. Men are at high risk for oral cancer, especially smokers and alcohol drinkers are more susceptible. Areca nut, often used with betel quid and chewable tobacco is the fourth most commonly used psycho-active substance in the world, ranking after caffeine, alcohol and nicotine. They share a common risk factor profile, including regular consumption of products of betel, areca and tobacco. Analysis of tumor markers in oral cancer is important to understand the severity of the disease. In this work, across-sectional study was carried out to understand the relation of socio-demographic factors with tobacco toxicity in part of Indian population. Sociodemographic variables of patients were noted on through various questionnaires of standard format. Serum α-feto protein (AFP), β_2-microglobulin (β_2m) and carcinoembryonic antigen (CEA) were analyzed before and after treatment with radiotherapy in oral cancer patients. The frequency of this cancer was found to be higher in the age of 45-60 years; People who chewed tobacco, spicy food with animal fat, poor oral hygiene, and low educational status were shown to have oral cancer. Lack of awareness of the risk factors for oral cancer leads to delay in diagnosis of the disease poor survival of the patients. With these considerations, the present study designed to determine the socio-demographic factors which are involved in oral cancer development by habitual use of the above products. Alterations in the levels of cancer markers in oral cancer patients were observed in different stages of oral cancer. The treatment efficacy shows the regression of the tumor in oral cancer patients. Systematic, high-quality and theory-driven research in this area is immediately needed to improve the healthcare of the patients.

KEY WORDS: Tobacco, oral cancer, alcohol, diet, radiation, tumor markers

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INTRODUCTION

Oral cancer (OCC) is the second most common cancer in men and the fourth most common cancer in women constitutes 13%–16% of all cancers[1]. The five - year survival rate is 75% of patients with local lesions, but only 17% of those with distant metastasis[2]. India has the highest prevalence of oral cancer in the world (19/100,000 population). Therefore, early diagnosis of oral cancer is important. Since the oral cavity is easily accessible for examination and the cancer is always preceded by some pre-cancerous lesion or condition such as a white or red patch, an ulcer or restricted mouth opening, it is preventable to a great extent. Unfortunately, in India, most cancers are diagnosed at a very late stage, it causes severe pain and treatment becomes more expensive, but the morbidity and mortality also increases. It is considered to be a disease of civilized society, related to lifestyle factors, but heredity also plays a role.Detecting oral cancer at an early stage is the most effective means of improving survival and reducing morbidity from this disease, yet a significant proportion of patients delay seeking help after the self-discovery of symptoms of oral cancer. Sociodemographic variables, and patient health-related behaviors and patient delay are the problems in developing oral cancer, and yet at present, the reasons for such delays are poorly understood and under-researched. Various measures of social support have also been inversely related to depression in head and neck patients[3]. Systematic, high-quality and theory-driven research in this area is immediately required. Chewing betel quid is a popular habit in tropical areas. It is also a known fact that oral cancer is one of the most common cancers in India, where betel quid chewing isprevalent[4]. Of all the oral cancers, 95% are related to the use of tobacco. This study deals with which sociodemographic variables are directly linked with carcinogenesis of oral cavity. In oral carcinoma, the study of biochemical tumor markers has been limited[5]. Several tumor markers [ferritin, N-acetyl neuraminic acid, phosphohexose isomerase, alpha-fetoprotein (AFP), β_2-microglobulin (β_2-m), carcinoembryonic antigen (CEA)] with clinical promise need further evaluation. Most of the AFP-producing oral cancer was advanced at the time of presentation, and AFP-producing early oral cancer is extremely rare. Serum AFP was thus found to be a reliable parameter in post-operative radiation and/or chemotherapy[6]. The serum β_2-m is in the free form and increased levels have been reported in patients with oral cancer as well[7-9], but there are only limited studies on β_2-m in oral cancer. CEA is the most widely studied tumor marker; CEA is an antigen that is preferentially associated with transformed tumor or cancer cells. Most of the clinical research on CEA, however, has focused on its use in monitoring the possible recurrence of cancer in patients who have had tumors removed.

MATERIALS AND METHODS

Patients presenting at the Government Arignar Anna Memorial Cancer Research Institute and Hospital, Kancheepuram, Tamil Nadu, India, were included in the present study and they were histologically confirmed. A total of 327 OCC patients (age mean ± SD=50 ±15 years): 202 males and 125 females, had cancer at various sites such as cheek (n=149), alveolus (n=42), tongue (n=61), floor of the mouth (n=28), lip (n=12), palate (n = 21), retro molar trigone (n=9) and combined sites (n=5). Tumor staging was, according to the TNM (Tumor Nodular Metastasis) classification of the UICC (International Union against Cancer) into stages: I (n=22), II (n=49), III (n=209) and IV (n=47). All people gave informed consent prior to the inclusion in the study. Questionnaires were used to investigate the sociodemographic features, smoking, tobacco chewing, alcohol consumptions, and dietary habits, family history of cancer, literacy, and income status of oral cancer patients. Studies were performed in accordance with the ethical standards of the Institution. All patients were clinically staged according to TNM staging system of the International union against cancer (UICC). Sixty oral cancer patients among 327 patients were randomly selected for tumor marker analysis. The present study groups were divided in to three groups ie. Group I-normal

healthy individuals (n=25; age mean ± SD=45±20 years)), group II-oral cancer patients who were further divided into four sub groups based on their stages. Group IIa: 8 patients with stage I, group IIb: 12 patients with stage II, group IIc: 27 patients with stage III, group IId: 13 patients with stage IV. Oral cancer patients treated with radiotherapy (Group III) were named into group IIIa (stage I), group IIIb (stage II), group IIIc (stage III) and group IIId (stage IV). Under aseptic precautions venous blood was drawn, serum separated from different stages of oral cancer patients before and after radiotherapy. The samples were frozen at-70°C until assay. Serum markers such as AFP, CEA, β_2-m was measured using ELISA plate micro well reader by standard procedures given in the kit. Tissue was collected by the dental surgeon in the affected area of the tumor before start of the treatment. These tissues were stored in the 10% formalin; sectioned tissue was stained with hematoxylin and eosin. The histology of the tissue was confirmed and interpreted by oral pathologist.

RESULTS

In an attempt to extend and update information relating oral cancer in the present clinical study provide a basis for comparison to other similar studies; a group of 327 patients with oral cancer has been studied in detail. The percentage of different demographic variables influencing oral cancer, various stages and grades are given in Table1and 2 respectively. Histological sections of the various tumor grades have been shown in Figure 2, 3, and 4.

Table 1

Different demographic variables of oral cavity Cancer patients in the study samples

Demographic variables	Total (n=327) (%)	Male (n=202) (%)	Female (n=125) (%)
Age			
< 45 years	14	14	14
45 – 60 years	60	56	66
>60 years	26	30	20
Income status			
Low income	85	87	86
Middle income	13	11	13
Higher income	2	2	1
Site of Occurrence			
Cheek	45	33	66
Alveolus	13	16	7
Tongue	18	23	11
Floor of the mouth	9	10	6
Lip	4	5	2
Palate	6	7	5
Retro molar trigone	3	4	2
Combined sites	2	2	1
Literacy status			
Illiterates	70	71	68
Primary level	21	20	22
Secondary level	7	6	8
College level	2	3	2
Diet			
Non – vegetarians	87	86	90
Vegetarians	13	14	10
Habits			
Chewing tobacco	51	30	81
Smoking	9	13	2
Alcohol	6	6	5
Alcohol+Smoking	9	14	-
Alcohol+Chewing	11	15	3
Smoking+Chewing	6	9	2
Smoking+Alcohol +Chewing	7	11	2
None	1	2	1
Oral Hygiene			
Poor(Charcoal powder, sand)	22	22	22
Fair (Paste+finger)	68	69	66
Good (Paste+brush)	10	9	12

Table 2
Stages and Histological grading of oral cavity cancer patients in the study samples

Stages	Total (n=327) (%)
Stage I – T_1 N_0 M_0	7
Stage II – T_2 N_0 M_0	15
Stage III – T_3 N_0 M_0, T_1 N_1 M_0, T_2 N_1 M_0	64
Stage IV – T_3 N_1 M_0	14

Figure 1

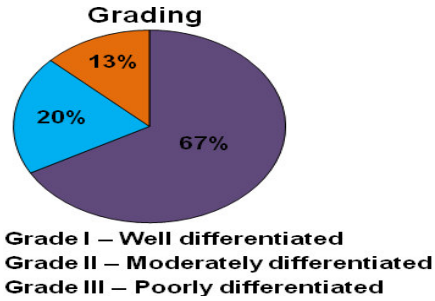
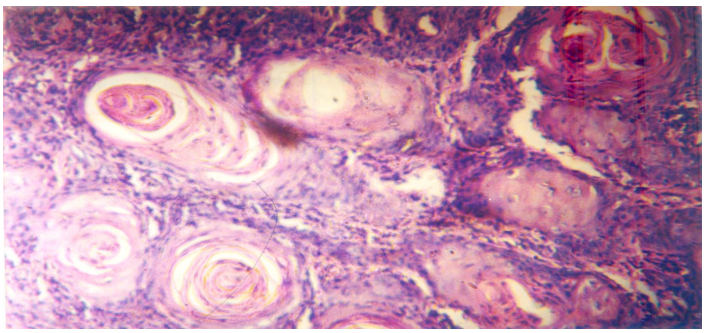
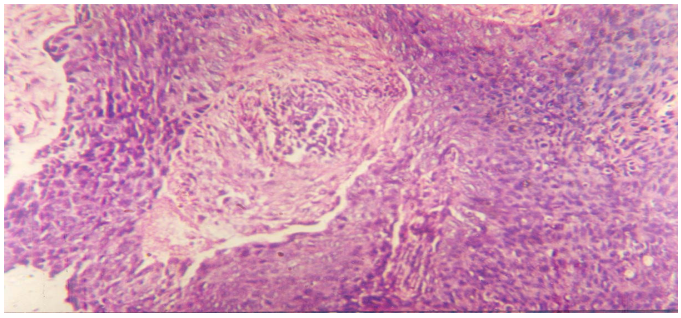


Figure 2
Well differentiated squamous cell carcinoma of buccal mucosa(Grade I)



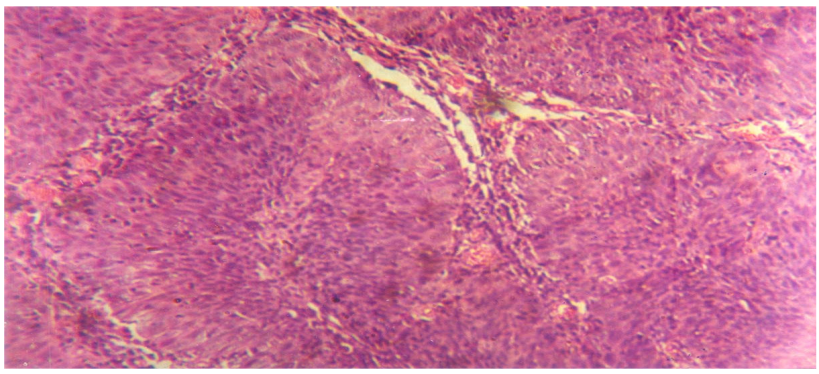
Closely packed nests of well differentiated carcinomatous cells with evidence of keratinization and formation of keratin pearls. Centre stroma is fibrous, scanty, richy vascular and infiltrated by inflammatory cells.

Figure 3
Moderately differentiated squamous cell carcinoma of buccal mucosa (Grade II)



Large irregular groups of polygonal epithelial cells containing less cytoplasm with hypochromatic pleomorphic central layers showing keratinizing malignant squamous cell.

Figure 4
Poorly differentiated squamous cell carcinoma of buccal mucosa (Grade III)



Large irregular groups of carcinomatous cell with nuclear and cytoplasmic features of malignancy. Very little differentiation and keratinization with increased mitotic activity.

The ratio of oral cancer patients from men to women was found as16:10. The frequency of oral cancer occurrence was high in the age of 45-60 years (60%) followed by the age of >60 years (26%). Lesser frequency was observed in the age of <45 years (14%). At all of the ages men were found to be predominant in developing oral cancer than women. Income status showed that 85% of oral cancer patients with low income followed by 13% of middle income range. Lesser percentage (2%) of oral cancer patients was observed among higher income range. Literacy status has inverse correlation with cancer vulnerability, showed that oral cancer was more prevalent among illiterates (70%). None of the patients had family history of any form of malignancy. Dietary pattern of the patients showed higher incidence among non-vegetarians (87%) and lesser among vegetarians (13%).Habits like chewing tobacco, smoking and alcohol alone and/or combined had profound effect on the incidence of oral cancer. Chewing tobacco alone had a greater impact contributing 51% of incidence. Smoking alone also appears to have significant effect as 9% reported oral cancer, whereas alcohol alone contributed 6%. Furthermore a combination of such habits like alcohol & smoking, alcohol & chewing, smoking & chewing, and smoking, alcohol & chewing showed 9%, 11%, 6% and &7% of oral cancer incidence respectively. 1% of oral cancer patients in the study group had none of the above mentioned habits. Oral hygiene appears to play a significant role in oral carcinogenesis. Patients with the fair hygiene status (68%) mostly developed oral cancer followed by poor hygiene (22%). 10% of patients with good oral hygiene developed oral cancer. The site of oral cancer showed that cheek was the predominant site (45%) followed by tongue (18%), alveolus (13%), floor of the mouth (9%), palate (6%), lip (4%), retro molar trigone (3%) and combined sites (2%). The occurrence of oral cancer in cheek and floor of the mouth were more predominant in women than in men, the incidence of oral cancer in all the other sites was higher. The clinical staging of these patients revealed that 34% of patients were in T_2, 44% in T_3 and 22% in T_1N_1. Most of these patients had well differentiated carcinoma. The mean values of serum AFP in oral carcinoma patients, after treatment with radiotherapy and controls are given in table 3. The elevated serum AFP concentration was observed in different stages of cancer (38±0.9 to 101.6±1.1 ng/mL) when compared to normal healthy individuals (3.8±0.6 ng/mL). In radiation treated groups show that the reduction in these levels (18.1±0.8 to 48.2±0.9) when compared to the respective stages of oral cancer (Untreated with radiation).

Table 3
Serum levels of α-fetoprotein in control, oral cancer and radiation treated oral cancer patients

Group	Clinical Condition (No. of patients)	Serum AFP Levels (ng/mL) (mean±SD)	Level of significance
Group-I	**Normal Healthy Individuals (n=25)**		
		3.8 ± 0.6	-
Group-II	**Oral cancer**		
Group-IIa	Stage-I (n=8)	38.9 ± 0.9	p<0.001
Group-IIb	Stage-II (n=12)	60.2 ± 1.3	p<0.001
Group-IIc	Stage-III (n=27)	92.8 ± 2.3	p<0.001
Group-IId	Stage-IV (n=13)	101.6 ± 1.1	p<0.001
Group-III	**Radiation treated oral cancer patients**		
Group-IIIa	Stage-I# (n=8)	18.1 ± 0.8	p<0.001
Group-IIIb	Stage-II# (n=12)	26.9 ± 1.0	p<0.001
Group-IIIc	Stage-III# (27)	40.2 ± 1.2	p<0.001
Group-IIId	Stage-IV# (n=13)	48.2 ± 0.9	p<0.001

Different stages of oral cancer patients were compared with normal healthy individuals. # - Different stages of radiation treated patients were compared with respective stages of oral cancer patients.

The mean values of serum β2-m in oral carcinoma patients, after treatment with radiotherapy and controls are given in table 4. In control (Group I), the mean β2-m value was 1.53±0.22 mg/L. The mean β2-m value was increased in different stages of oral cancer (2.09±0.43 to 3.52±0.53 mg/L) patients when compared with control. In radiation treated patients these levels were significantly reduced in the range between 1.89±0.13 to 3.12±0.23 mg/L when compared to different stages of oral cancer patients (radiation untreated).

Table 4
Serum levels of β_2-microglobulinin control, oral cancer and radiation treated oral cancer patients

Group	Clinical Condition (No. of patients)	Serum β_2-microglobulin (mg/L) (mean ± SD)	Level of significance
Group-I	**Normal Healthy Individuals (n=25)**		
		1.53 ± 0.22	
Group-II	**Oral cancer**		
Group-IIa	Stage-I (n=8)	2.09 ± 0.43	p<0.001
Group-IIb	Stage-II (n=12)	2.33 ± 0.39	p<0.001
Group-IIc	Stage-III (n=27)	2.83 ± 0.32	p<0.001
Group-IId	Stage-IV (n=13)	3.52 ± 0.53	p<0.001
Group-III	**Radiation treated oral cancer patients**		
Group-IIIa	Stage-I# (n=8)	1.89 ± 0.13	p>0.05
Group-IIIb	Stage-II# (n=12)	2.01 ± 0.09	p<0.05
Group-IIIc	Stage-III# (27)	2.39 ± 0.10	p<0.001
Group-IIId	Stage-IV# (n=13)	3.12 ± 0.23	p<0.05

Different stages of oral cancer patients were compared with normal healthy individuals. # - Different stages of radiation treated patients were compared with respective stages of oral cancer patients.

The mean values of serum CEA in oral carcinoma patients, after treatment with radiotherapy and controls are given in table 5.In the controls (Group I) the mean CEA value was 1.5±0.5ng/mL. The mean CEA value is showed from 2.5±0.3 to 5.9±0.5mg/mL in different stages of oral patients. In radiation treated patients these levels were significantly reduced in the range of 1.4±0.9 to 4.2±0.6 mg/mL when compared to different stages of oral cancer patients (radiation untreated).

Table 5
Serum levels of Carcinoembryonic antigen in control, oral cancer andradiation treated oral cancer patients

Group	Clinical Condition (No. of patients)	Serum CEA (mean ± SD)	Level of significance
Group-I	**Normal Healthy Individuals (n=25)**		
		1.5 ± 0.5	-
Group-II	**Oral cancer**		
Group-IIa	Stage-I (n=8)	2.5 ± 0.3	p<0.001
Group-IIb	Stage-II (n=12)	3.8 ± 0.6	p<0.001
Group-IIc	Stage-III (n=27)	5.1 ± 0.8	p<0.001
Group-IId	Stage-IV (n=13)	5.9 ± 0.5	p<0.001
Group-III	**Radiation treated oral cancer patients**		
Group-IIIa	Stage-I# (n=8)	1.4 ± 0.9	p<0.01
Group-IIIb	Stage-II# (n=12)	2.9 ± 0.9	p<0. 01
Group-IIIc	Stage-III# (27)	3.6 ± 0.4	p<0.001
Group-IIId	Stage-IV# (n=13)	4.2 ± 0.6	p<0.001

Different stages of oral cancer patients were compared with normal healthy individuals. # - Different stages of radiation treated patients were compared with respective stages of oral cancer patients.

DISCUSSION

In the present study, cancer lesions were predominant in middle aged and older people than younger people. This cancer incidence might be due to high cumulative exposure, and carcinogenesis is a prolonged process that may take several years to manifest, so the increased occurrence in the age of 45-60 years may be due to the increased susceptibility of older tissues with suppressed immune status[10,11] to environmental carcinogenesis than younger tissues. Higher incidence among the low income group might be due to increased exposure to UV light[12] and saw dust, as most of the patients were farmers, hardwood furniture manufacturers[13].Primitive ideas on oral hygiene and oro-dental diseases may be the reason for illiterates to contribute a major part in OCC incidence[14].Lesser percentage of literates also was reported OCC, as the habit of chewing/smoking tobacco was not only seen among uneducated and underprivileged, but also among educated. Widespread consumption of tobacco may be the reason of OCC[15].Genetic predisposition to OCC has been reported[16,17] whereas opposed by Mackenzie *et al*[18]. In the present study, no occurrence of cancer due to hereditary. The increased incidence among non-vegetarians may be specified at increased exposure to spices than vegetarians. Spices like cardamom, cloves, aniseed induce oral cancer[19].The habitual intake of foods rich in animal and saturated fat: pork meat, cheese, soups and fried food leads to the predisposition of oral cancer incidence[20].These findings suggest that there is a non-negligible scope for the prevention of oral cancer through the improvement of diet. Illiteracy, gender, dietary habits, having poor oral hygiene and denture sores is associated with primary oral cancer in this patient sample; but eating vegetables were related with healthy status. Determination of the factors associated with oral cancer and of the high-risk groups would be beneficial to provide efficient screening protocols and prevention programs for oral cancers. The overall higher incidence of the cancer in cheek and alveolus in the present study may be attributed to increased habit of chewing tobacco. The prevalence of this habit was evidently higher among men (30%) and women (81%) and this may have increased the overall higher incidence of cancer in cheek. Smokeless tobacco in the form of dipping and chewing or as quid with betel nut and tobacco provides carcinogenic stimulus[21,22]. Efforts to reduce habitual betel quid consumption and smoking might be of benefit in reduction of oral cancer incidence. The incidence of cancer in tongue and lip may be related to the habit of smoking. The increased habit of smoking

among men (13%) may have contributed to an increased incidence of these cancers among men[23].As smoking was less prevalent among women (2%). Alcohol intake also plays a vital role in the incidence of cancer in lateral border and ventral surface of the tongue and the floor of the mouth. The combination of these habits appears to play a major role in carcinogenesis as 7%, developed oral cancer in both men and women population in the present study. Tobacco- smoked, chewed and inhaled increases the risk of pre-cancerous lesions[24]. Pan and alcohol have been established as the major risk factors for oral cancer. Irritant factors like excessive use of mouth wash, jagged teeth, ill-fitting denture, and sharp overhanging or jacket crown might have acted as a promoter of carcinogenesis in patients with none of the habits. Oral hygiene plays a major role in the development of oral cancer. Poor oral hygiene seems to be an independent risk factor in the development of oral cancer[25].Patients using brush and tooth paste for cleaning purpose had a better oral hygiene and so a reduced oral cancer incidence was observed in these patients. Patients using powder and finger were found to be associated with higher prevalence of oro-dental disorders. This may be due to the influence of the oral acidogenic micro flora such as *Streptococcus mutants, Lactobacillus* and *Candida*, human papilloma virus infection which are the factors that may influence the oral carcinogenesis[26]. Localization of AFP was confirmed in the cancer cells. However, AFP one of the onco-fetal antigens, has never been implicated in salivary glands[27]. Our results are consistent with these data. AFP has been considered as a stage-specific antigen. The positive reactions we observed might be stage-specific. Serial AFP estimations help in distinguishing nonmalignant and malignant conditions, as the steady and progressive rise of AFP is observed in malignancies whereas nonmalignant conditions show fluctuations and transient moderately elevated concentrations[28,29]. Increased serum AFP concentration was noticed in case of endodermal sinus tumor of nasopharynx in 4 year old child[30]. Measurement of serum AFP has also been useful in monitoring efficacy of chemotherapy, surgery and radiotherapy in primary hepatocellular carcinoma, hepatoblastoma, non-seminomatous testicular and other germ cell tumors[31]. A close correlation between serum AFP and progression or regression of tumor was observed in the present study. It was observed that there was a significant rise in serum β_2-min oral carcinoma patients. Production of onco-fetal antigens such as carcinoembryonic antigen has been well documented in salivary glands. Progressively higher values were obtained as the cancer advanced clinically. The previous report suggests that the levels of glycoconjugates are also considered as one of the diagnostic tests for oral cancer and effectiveness of radiotherapy[32]. Therefore the estimation of serum β_2-m may be useful as one of a battery of tests in the assessment of oral carcinoma patients[33]. The possible role of β_2-m as a biochemical parameter and its significance in oral cancer has been discussed[34].The mechanism in which increase in β_2-m levels in malignancies is not known but various possible hypotheses for the increased serum levels have been put forward. However, due to its non-specificity and its moderate elevation is observed in case of solid tumors and also in various inflammatory diseases, benign infectious disorders etc. it is used routinely for evaluating tumor cell load, disease activity and prognosis. It is also used to monitor efficacy of patient's response to treatment[28]. The β_2-m is a content of cell membrane along with the HLA chain, so an accelerated membrane turnover or accelerated cell division could increase the shedding of β_2-m[35]. β_2-m has proved to be the best marker for monitoring therapeutic course, as it is useful serum parameter to monitor tumor progression as well as early biochemical relapse[28]. Hence further studies are necessary to find out whether serum β_2-m would be of help in clinical diagnosis and also effectiveness of radiation treatment. The results of clinical studies states that CEA, although originally thought to be specific for digestive tract cancers, may also be elevated in other malignancies and in some nonmalignant disorders. Persistently rising CEA value may be associated with progressive malignant disease

and a poor therapeutic response. A declining CEA value is generally indicative of a favorable prognosis and a good response to treatment[28]. Initial CEA levels were elevated in cancer patients, the incidence varying with stage of disease. Greater than 2.5 ng/mL may be associated with cancer, in particular those of gastrointestinal tract, pancreas, ovary, lung and breast.CEA values are not specific for cancer, although very high levels (e.g. >20mg/ml) are highly suggestive of malignancy[36]. It is important that serial assays of CEA are used in reaching a clinical judgment, and not any single determination. Radiation therapy of localized colorectal cancer reliably reduced the elevated circulating CEA titers with accumulating doses of irradiation may indicate that the bulk of CEA-producing tumor is within the radiation treatment portal. The decrease of circulating CEA with preoperative radiation therapy was short lived and suggested that surgical resection should be performed within 8 weeks of irradiation[37]. Apart from clinical features, socio-demographic factors also significantly influenced the survival of oral cancer patients. Therefore, care providers should take socio-demographic issues into consideration aside from ordinary clinical health care. Healthcare factors and psychosocial factors may play a role, but the research in this area is sparse, a theoretical and of poor quality. Patient delay in diagnosis is a problem in oral cancer and yet at present the reasons for such delays are poorly understood and under-research. These cancer markers may help to elucidate the effect of radiation treatment in cancer along with other demographic factors.

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